Concise report

Laser Doppler perfusion imaging in systemic sclerosis impaired response to cold stimulation involves digits and hand dorsum

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Abstract

Objectives. To assess by Laser Doppler perfusion imaging (LDPI) skin blood perfusion of hands in patients with SSc and primary RP (PRP) at baseline and after cold stimulation (CS). In SSc patients, the associations between skin perfusion and nailfold video capillaroscopy (NVC) patterns were also evaluated.

Methods. Forty patients with SSc, 38 patients with PRP and 32 healthy controls were recruited. Skin blood flow of the hands was detected by Lisca Laser Doppler Perfusion Imager at baseline and after CS. Further laser Doppler scanning was performed for each hand at 0 (T₁), 3 (T₂), 7 (T₃) and 15 min (T₄).

Results. Baseline mean perfusion is significantly (P < 0.0001) lower in SSc patients than in healthy controls. In SSc patients, mean perfusion is reduced after CS (P < 0.0001) and skin flow recovery (significant difference between T₀ and T₄, P < 0.0001) is incomplete. In SSc patients with low vascular damage (early and active capillaroscopic groups), the abnormal microvascular response to CS involves only the digits, while the perfusion of hands dorsum is normal. With the progression of vascular damage (late capillaroscopic groups), the abnormal microvascular response to CS also appears in the hand dorsum skin. In PRP patients, baseline hand perfusion is very low and the skin flow recovery after CS is absent (P < 0.05).

Conclusion. In early SSc, the thermoregulation of finger skin is impaired, but only in advanced stages of microangiopathy does the skin of the hand dorsum show a vasomotor control failure.

Key words: Systemic sclerosis, Raynaud’s phenomenon, Skin blood perfusion, Laser Doppler perfusion imaging, Cold stimulation.

Introduction

SSc is a CTD characterized by endothelial dysfunction and fibrosis of the skin and internal organs [1]. Microangiopathy is one of the main histopathological features that is detectable early in the course of the disease [2]. SSc is typically associated with RP. RP secondary to SSc is characterized by microvascular damage.

In contrast to SSc, primary RP (PRP) does not progress to irreversible microvascular injury [3]. Nailfold video capillaroscopy (NVC) is the most reliable way to distinguish between PRP and secondary RP by identification of an early pattern of SSc [4]. Previous studies by laser Doppler perfusion imaging (LDPI) demonstrate the key role of ischaemia in SSc skin lesions [5–7]. A relationship between the impaired blood perfusion at fingertips, analysed by laser Doppler flowmetry (LDF), and the morphological microvascular abnormalities detected by NVC, was observed in SSc patients [8]. Our aim was to assess skin blood perfusion by LDPI at baseline and after cold test and its relationship with NVC patterns in SSc patients compared with healthy controls and PRP patients.
Patients and methods

Patients

Forty patients [32 females and 8 males; median age 45 (range 26–65) years, median duration of RP 14 (range 3–30) years and median duration of SSC 9 (range 2–26) years] fulfilling the ACR criteria for the classification of SSC were enrolled in this study [9]. Twenty patients had lcSSc and 20 had dcSSc as defined by LeRoy et al. [10]. Eighteen patients had a history of digital ulcers. Eighteen patients were ACA positive and 14 were anti-topoisomerase antibody positive.

SSc patients with active digital ulcers were excluded from this study. None of the patients had received treatment with parenteral prostanoids or bosentan for at least 3 months before this study point. Patients in current therapy with prostacyclin analogues, phosphodiesterase 5 inhibitors and endothelin receptor antagonists, prior sympathectomy of the upper limb and with a history of uncontrolled systemic hypertension, hyperlipidaemia, cardiac failure, hepatic failure, diabetes, peripheral vascular diseases, coagulopathy and smokers, pregnant or breastfeeding women were excluded.

Thirty-eight patients with PRP [29 females and 9 males, median age 32 (range 20–62) years, median duration of RP 13 (range 4–33) years] and 32 healthy controls [24 females and 8 males, median age 37 (range 20–68) years] were also recruited [11].

SSc and PRP patients underwent therapeutic treatment with calcium channel blockers (nifedipine 30 mg/day). Median duration of therapy was 11 (range 2–28) and 9 (range 3–29) years, respectively. Therapy was discontinued 48 h before the examination according to our previous study [5]. The subjects’ written consent was obtained according to the Declaration of Helsinki and the study was approved by the ethics committee of Sapienza University.

LDPI and NVC

LDPI and NVC were performed after resting the subject in a temperature-controlled room at 24 (0.4) °C for 20 min. Patients and healthy controls did not drink alcoholic beverages or coffee for 2 days before the examination. According to our previous study, SSc patients with marked sclerodactyly were excluded [5]. The investigator who analysed the LDPI was blinded for NVC findings.

LDPI assessment

Baseline (T₀) images were taken of the dorsal aspects of both hands with a low-energy 670 nm Lisca Laser Doppler Perfusion Imag (Perimed AB, Stockholm, Sweden). LDPI images were taken at a distance of 15 cm, giving an area of 12 × 12 cm. All perfusion signals have been combined to form a colour-coded image using a scale ranging from dark blue (lowest value) to red (highest value). The term commonly used to describe the blood flow measurements, by the laser Doppler technique, is flux. Flux has been expressed by arbitrary perfusion units (pU), which are directly proportional to the product of the mean speed and the concentration of red blood cells. For each image, the instrument gives the numerical parameters (pUs): minimum perfusion, mean perfusion and maximum perfusion.

After baseline blood flow measurement, patients and healthy controls underwent a cold stimulus (CS). The CS was performed using a box, cooled and ventilated, at a temperature of 4 °C (Microlab Elettronica Sas, Pordenone, Italy). Participants underwent CS by placing their hands in the box (4 °C) for 5 min. Hands were imaged for 15 min after CS. Blood flow measurements were made at 0 (T₁), 3 (T₂), 7 (T₃) and 15 min (T₄).

According to our previous study, the dorsum of the hand was divided into three regions of interest (ROI): ROI1, ROI2 and ROI3. ROI1 included three fingers of the hand from the second to the fourth distally to the proximal interphalangeal finger joint. ROI2 included the area between thePIP and the MCP joint. ROI3 included only the dorsal surface of the hand without the fingers. A proximal–distal gradient was present when the perfusion mean difference between ROI1 and ROI2 was > 0.3 pU [5].

NVC

NVC was performed with a videocapillaroscope (Pinnacle Studio Version 8) equipped with a 500 × optical probe. The nail-fold of the second, third, fourth and fifth finger was examined in each patient. According to Cutolo et al. [12], the patterns identified within the SSc pattern include: early, active and late.

Statistical analysis

All the results were expressed as median and range. Commercially available software (SPSS version 18.0) was used for statistical analysis. Chi-square test was used for categorical variables. The coefficient of skewness and coefficient of kurtosis were used to evaluate normal distribution of data. The assumptions of normality cannot be assumed within the data set particularly due to the small sample size; therefore, non-parametric tests were used throughout. The Mann–Whitney U test was used to test differences between two individual study groups. Wilcoxon signed-rank test was used to compare results at different time points. Longitudinal comparison among successive time points was analysed by repeated-measures analysis of variance (ANOVA). Spearman’s rank order correlation (R) and Pearson’s product–moment correlation coefficient (r) were used to test for an association between age or disease duration and numerical LDPI parameters. P < 0.05 was considered statistically significant.

Results

At baseline the proximal–distal perfusion gradient is present (P < 0.003) in a high percentage of healthy controls (94%), while it is observed in a low percentage of SSC (12%) and PRP (5%). In three study groups no significant change from baseline was observed during CS in proximal–distal perfusion gradient.
In SSc patients, mean perfusion was significantly lower than that in healthy controls at baseline and also at all time points after CS. In healthy controls, mean perfusion was reduced after CS \( (P < 0.0001) \) and it returns to initial values after CS (no significant difference between \( T_0 \) and \( T_4 \), \( P > 0.05 \)). In SSc patients, mean perfusion is reduced after CS \( (P < 0.0001) \), while the skin flow recovery (significant difference between \( T_0 \) and \( T_4 \), \( P < 0.0001 \)) is incomplete. In PRP patients, mean perfusion shows significant changes from baseline \( (P < 0.01) \); instead the skin flow recovery (significant difference between \( T_0 \) and \( T_4 \), \( P < 0.05 \)) is incomplete (Fig. 1).

In SSc patients at baseline, the mean perfusion of ROI1 shows lower values than in healthy controls, but higher than in PRP patients \( (P < 0.001) \). In healthy controls and PRP patients, the perfusion curves of three ROIs show the same trend; conversely, the perfusion of SSc patients differs in three ROIs. In SSc patients, the mean perfusion of ROI1 and ROI2 at baseline and after CS is impaired, whereas the perfusion curve of ROI3 is normal. Conversely, in PRP patients perfusion curves show no significant differences in the three ROIs (Fig. 1).

Fifteen patients have an early capillaroscopic pattern, 14 have an active capillaroscopic pattern and 11 have a late capillaroscopic pattern. The SSc patients with active pattern show a mean perfusion at baseline higher \( (P < 0.001) \) than the other two capillaroscopic groups (Fig. 2). In SSc patients with early capillaroscopic pattern, mean perfusion is reduced after CS \( (P < 0.0001) \) and it returns to basal values during recovery (non-significant difference between \( T_0 \) and \( T_4 \), \( P > 0.05 \)). In SSc patients with active capillaroscopic pattern, mean perfusion is reduced after CS \( (P = 0.001) \), while the perfusion recovery (significant difference between \( T_0 \) and \( T_4 \), \( P < 0.01 \)) is incomplete. In SSc patients with late capillaroscopic pattern, mean perfusion does not significantly decrease after CS \( (P = 0.05) \), but the skin flow recovery (significant difference between \( T_0 \) and \( T_4 \), \( P = 0.05 \)) is absent (Fig. 2).

Perfusion curves of the three capillaroscopic groups are different in the three ROIs. In the three capillaroscopic groups, the perfusion curves of ROI1 and ROI2 do not show a significant recovery at different time points of CS. Conversely, the perfusion curves of ROI3 show significant changes in the three capillaroscopic groups: patients with early and active capillaroscopic patterns showed normal recovery of skin flow after CS (no significant difference between \( T_0 \) and \( T_4 \), \( P > 0.4 \)), the skin flow recovery is absent in patients in the late capillaroscopic group (Fig. 2). There was no correlation between age or disease duration or disease subset or history of digital

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**Fig. 1** Laser Doppler Perfusion Imaging in patients and healthy controls. (A) Mean perfusion (median and 95% CI) in healthy controls and SSc and PRP patients. (B–D) Mean perfusion of three ROIs in three groups of study.
Discussion

Microvascular dysfunction is one of the hallmarks of SSc and is already present in early disease [13]. Skin blood flow depends on cutaneous microcirculation. Several techniques are currently used to assess the role of ischaemia in SSc skin lesions: NVC, LDPI thermal infrared imaging, digital photoplethysmography, colour Doppler ultrasonography, angiography and magnetic resonance angiography [14–16].

In SSc patients, baseline perfusion is lower than in healthy controls. In SSc patients, the perfusion curve of hand dorsum is different from that of the fingers and it has a trend similar to that of the healthy controls. In SSc patients, the CS response demonstrates that the sclerodermic microangiopathy is mediated by both microvascular damage and dysfunction. The baseline changes in LDPI parameters are mediated by microvascular damage, while the abnormal response to CS is due to microvascular dysfunction. Murray et al. [7] observed lower baseline perfusion and lower perfusion curve in patients with SSc as compared with healthy controls. Also Correa et al. [17] demonstrate that LDPI showed lower digital blood flow in SSc patients when compared with healthy controls at baseline and after CS.

Our findings showed that in the early stage of SSc microangiopathy (early and active capillaroscopic groups), the perfusion curve of hand dorsum is similar to that of healthy controls. In SSc patients with late capillaroscopic pattern, recovery after CS is absent in all three skin regions of hands. Murray et al. [18] suggest that SSc has no effect on microvascular perfusion in the dorsum of the hand, and that the abnormal microvascular response is localized to the digits, affecting both smaller and larger vessels. Conversely, the results of our study demonstrate that in the late stage of SSc, abnormal microvascular response to CS is localized also to the hands dorsum. Since in our study the perfusion curves of hand dorsum are different in the three sclerodermic capillaroscopic groups, we can suppose that SSc microangiopathy of hand dorsum appears late. Structural and functional changes in microcirculation appear early on skin regions corresponding to fingers. With the progression of vascular
damage (late capillaroscopic pattern), the structural and functional changes also appear in skin of hand dorsum [19].

In PRP patients, baseline hand perfusion is too low and skin flow recovery after CS is absent. In PRP patients, the modifications of the perfusion curves are only due to a dysfunction of the autonomic nervous system. Grattagliano et al. [20] suggest that PRP have a distinct pattern at LDF evaluation at baseline and after CS, and monitoring patients with PRP could be helpful in understanding whether a change in the LDF pattern might predict the development of SSc [20]. In conclusion, the skin perfusion of fingers is impaired early for the presence of both structural and functional changes of microcirculation, while in the hand dorsum the skin blood flow is impaired only in advanced stages of SSc microangiopathy.

### Rheumatology key messages

- In SSc patients, baseline skin perfusion and thermoregulation of fingers are impaired early.
- Skin perfusion and thermoregulation of hand dorsum are impaired in late stages of microangiopathy.

### Disclosure statement

The authors have declared no conflicts of interest.

### References